VOLUME 34 · NUMBER 25 · SEPTEMBER 1, 2016

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy

Sébastien Héritier, Jean-François Emile, Mohamed-Aziz Barkaoui, Caroline Thomas, Sylvie Fraitag, Sabah Boudjemaa, Florence Renaud, Anne Moreau, Michel Peuchmaur, Catherine Chassagne-Clément, Frédérique Dijoud, Valérie Rigau, Despina Moshous, Anne Lambilliotte, Françoise Mazingue, Kamila Kebaili, Jean Miron, Eric Jeziorski, Geneviève Plat, Nathalie Aladjidi, Alina Ferster, Hélène Pacquement, Claire Galambrun, Laurence Brugières, Guy Leverger, Ludovic Mansuy, Catherine Paillard, Anne Deville, Corinne Armari-Alla, Anne Lutun, Marion Gillibert-Yvert, Jean-Louis Stephan, Fleur Cohen-Aubart, Julien Haroche, Isabelle Pellier, Frédéric Millot, Brigitte Lescoeur, Virginie Gandemer, Christine Bodemer, Roger Lacave, Zofia Hélias-Rodzewicz, Valérie Taly, Frédéric Geissmann, and Jean Donadieu

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on July 5, 2016.

Written on behalf of the GENEHISTIO Study Group.

Supported by a grant from InVS and Institut National de la Santé et de la Recherche Médicale for the rare disease registry; this project received constant, unlimited support from the Association Histiocytose France; a grant from the Association Recherche et Maladie Hématologiques de l'Enfant; a grant from the Association Les 111 des Arts de Paris; a grant from the Association la Petite Maison dans la Prairie; a grant from the Gardrat family; and a grant from the Société Française de lutte contre les Cancers de l'Enfant et de l'Adolescent, the Fédération Enfants et Santé.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Corresponding author: Sébastien Héritier, MD, French Reference Center for Langerhans Cell Histiocytosis, Trousseau Hospital, 26 Ave du Dr Netter, 75012 Paris, France; e-mail: sebastien.heritier@trs.aphp.fr.

0732-183X/16/3425w-3023w/$20.00

DOI: 10.1200/JCO.2015.65.9508

ABSTRACT

Purpose
Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasia with a broad spectrum of clinical manifestations and outcomes in children. The somatic *BRAF*^{V600E} mutation occurs frequently, but clinical significance remains to be determined.

Patients and Methods
BRAF^{V600E} mutation was investigated in a French LCH cohort. We analyzed associations between mutation status and clinical presentation, extent of disease, reactivation rate, response to therapy, and long-term permanent sequelae.

Results
Among 315 patients with successfully determined *BRAF* status, 173 (54.6%) carried a *BRAF*^{V600E} mutation. Patients with *BRAF*^{V600E} manifested more severe disease than did those with wild-type *BRAF*. Patients with *BRAF*^{V600E} comprised 87.8% of patients (43 of 49) with multisystem LCH with risk organ involvement (liver, spleen, hematology), 68.6% of patients (35 of 51) with multisystem LCH without risk organ involvement, 43.9% of patients (86 of 196) with single-system LCH, and 42.1% of patients (8 of 19) with lung-involved LCH ($P < .001$). *BRAF*^{V600E} mutation was also associated with organ involvement that could lead to permanent, irreversible damage, such as neurologic (75%) and pituitary (72.9%) injuries. Compared with patients with wild-type *BRAF*, patients with *BRAF*^{V600E} more commonly displayed resistance to combined vinblastine and corticosteroid therapy (21.9% *v* 3.3%; $P = .001$), showed a higher reactivation rate (5-year reactivation rate, 42.8% *v* 28.1%; $P = .006$), and had more permanent, long-term consequences from disease or treatment (27.9% *v* 12.6%; $P = .001$).

Conclusion
In children with LCH, *BRAF*^{V600E} mutation was associated with high-risk features, permanent injury, and poor short-term response to chemotherapy. Further population-based studies should be undertaken to confirm our observations and to assess the impact of BRAF inhibitors for this subgroup of patients who may benefit from targeted therapy.

J Clin Oncol 34:3023-3030. © 2016 by American Society of Clinical Oncology

INTRODUCTION

Langerhans cell histiocytosis (LCH) is the most common type of histiocytosis and is characterized by inflammatory lesions with abundant $CD1a^{+}CD207^{+}$ histiocytes, which provoke the destruction of affected tissues. This disease most commonly affects children.[1,2]

Clinical behavior of LCH is remarkably heterogeneous; some cases are limited, indolent, and self-regressive, whereas others recur sequentially, are refractory to conventional therapy, and exhibit systemic and sometimes life-threatening multiorgan involvement. The severe clinical form of the disease principally affects young children (age < 2 years) and tends to involve risk organs (RO), including the hematopoietic system, spleen,

and/or the liver. Despite a low mortality rate,[3,4] long term, irreversible adverse effects are common.[5] In particular, endocrine dysfunction, secondary to pituitary involvement, and neurodegenerative disease are reported in approximately 20% and 5% of cases, respectively.[6] Currently, the molecular mechanisms that underlie these different LCH subtypes remain poorly understood.

Since 2010, LCH has been known to harbor the *BRAF*^{V600E} activating mutation in 38% to 64% of all cases.[7-10] Experiments conducted in a mouse model have suggested that this mutation may be mitogenic for dendritic cells.[7] This hypothesis was corroborated when a BRAF inhibitor demonstrated efficacy in LCH[11,12]; however, to date, the frequency of this mutation has only been assessed in small, unrepresentative patient samples.

The current study includes a cohort of children with LCH who were enrolled in the national French registry. We conducted careful analyses of this patient cohort to determine correlations between *BRAF*^{V600E} mutation status and clinical manifestations of LCH in their entirety.

PATIENTS AND METHODS

Patients and Sample Collection

Of 1,747 patients with childhood LCH (age < 18 years) who were included in the French LCH registry[6,13,14] (from 1983 to 2015), 399 patients had biopsy samples available and were contacted to participate in this study (Fig 1). Some biopsies were unavailable as a result of the destruction of samples after 10 years of banking, and some samples had damaged DNA because of preservation with Bouin's fixation. This study was approved by the Ethics Committee, Ile de France III (#2011-A00447–34) and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. Comprehensive descriptions of the experimental plan, sample size, and study organization are given in the Appendix (online only).

Demographic data, type of treatment, clinical characteristics, and extent of disease were recorded according to classifications established by the Histiocyte Society.[1] Patients were classified according to the Histiocyte Society LCH IV guidelines, which consider the number of organs—or systems—involved, lung involvement, and risk organ (RO) involvement. Relevant ROs were liver, spleen, and hematologic system. The four classifications were: single system (SS), that is, one organ and no lung or RO involvement; multiple system (MS) involvement, but no lung or RO involvement (RO−); lung involvement (Lung+), but no RO involvement; and MS involvement and at least one RO involved (RO+). Disease extent was evaluated at the time of initial diagnosis and at the time of maximal disease in instances of reactivation. Follow-up data were prospectively recorded according to previously described methodologies.[13,15]

Permanent consequences (PC) were defined as any irreversible clinical condition that developed at any time during the course of disease that could be directly attributed to the natural history of LCH or its treatment.[4] The Disease Activity Score (DAS)[16] was a quantitative score on the basis of clinical and biologic evaluations and was used to determine severity of disease for each patient.

Reactivation was defined as the reappearance of signs and symptoms of active disease after either complete disease resolution or a period of disease control that persisted for > 3 months on maintenance therapy. Treatment efficacy was evaluated according to the classification used by the Histiocyte Society.[4,17] Of 315 patients, 161 (51.1%) were treated with a systemic chemotherapy, which included, for all but five patients, a vinblastine-steroid combination therapy. The remaining five patients received a systemic therapy with vinblastine or 6-mercaptopurine monotherapy or a vinblastine-etoposide regimen. The vinblastine-steroid combination therapy included an initial 6-week course of vinblastine 6 mg/m^2 intravenous bolus weekly and continuous oral prednisolone (as per LCH-II and LCH-III trials and national guidelines).[18] All patients were evaluated for response to treatment at 7-week intervals. Patients who responded to therapy were defined as those who showed either complete resolution or continuous disease regression.[4,17]

Identification of the BRAF^{V600E} *Mutation*

Child patients with parents who were included in the French cohort and who had an available biopsy sample were contacted. Signed informed consent was obtained for 399 patients. For the majority of patients (76%), *BRAF* status was obtained in an ISO 15189-certified laboratory. For 306 patients, *BRAF*^{V600E} status was successfully determined with sequencing analyses. In brief, macro dissection was performed to obtain an infiltrate of CD207$^+$ histiocyte cells, which comprised > 20% of the cell population. *BRAF*^{V600E} mutation was detected by performance of pyrosequencing[10,19] with PyroMark Q24 (n = 261; Qiagen, Valencia, CA) or by performance of real-time polymerase chain reaction (n = 16; LightCycler 480; Roche, Basel, Switzerland). When histiocytes were a minor component of

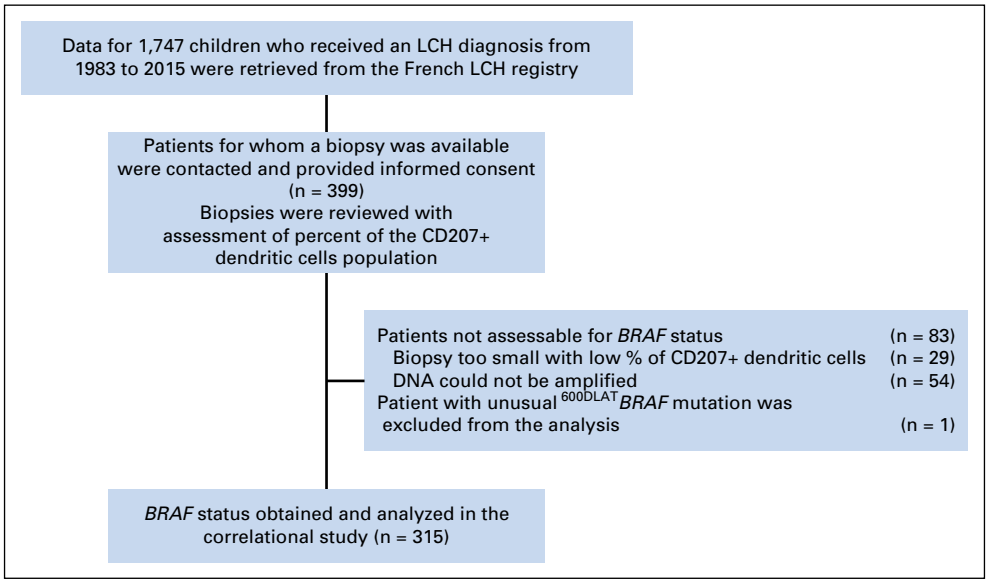

Fig 1. Study cohort selection. LCH, Langerhans cell histiocytosis.

infiltrate, that is, < 20% of the cell population, and sequencing produced a negative result, or when histiocyte infiltration dipped beneath 10%, we performed a droplet digital polymerase chain reaction assay (n = 29) with a Raindrop system (Raindance Technologies, Billerica, CA).[20] In nine patients, immunochemistry was performed by staining histiocytes with the mouse monoclonal antibody, VE1.[10] Sixteen patients had been previously reported by Satoh et al.[9] We failed to determine *BRAF* status in 83 patients (20.8%) either because of a small sample with a low percentage of $CD1a^{+}$ histiocytes (n = 29) or a failure in DNA amplification (n = 54). Finally, the patient with ^{600DLAT}*BRAF* mutation previously reported[9] was excluded from the present analysis.

Statistical Analyses

Differences between groups were tested with the Mann-Whitney *U* test for quantitative variables and with Fisher's exact test for qualitative variables. For statistical analysis, threshold significance was .01, and for univariable analyses of LCH presentation according to *BRAF* status—because multitests were performed—$P < .002$ was considered statistically significant (Bonferroni correction). Multivariable binary logistic regression analyses were performed to calculate odds ratios (ORs) and 95% CIs to identify significant features associated with *BRAF* mutational status. End points for survival analyses were any type of reactivation. Survival analyses included the interval between diagnosis and an event (reactivation or death) or the last examination. Survival rates were estimated by using the Kaplan-Meier method, and subgroups were compared with the log-rank test. All statistical analyses were performed with STATA 13 software (STATA, College Station, TX; Computing Resource Center, Santa Monica, CA). Cutoff date for these analyses was October 30, 2015. Eight patients received targeted therapy with a BRAF inhibitor, and date of last follow-up was censored on the day the first dose was administered.

RESULTS

BRAF somatic status was obtained for 315 children who were diagnosed with LCH. This cohort comprised 167 (53%) boys and 148 (47%) girls. Patient clinical characteristics (Table 1) were comparable between the study cohort and 1,431 children who were not investigated for *BRAF* status but were included in the LCH registry from 1983 to 2015; however, these groups had different follow-up durations. The study cohort had shorter a follow-up than did the uninvestigated cohort (median, 3.2 and 4.4 years, respectively; $P = .003$).

Median age of the study cohort at diagnosis was 3.2 years (range, 0 to 17.9 years). The 315 patients were classified as follows: 196 (62.2%) patients with SS LCH, 51 (16.2%) with MS RO− LCH, 19 (6.0%) with Lung+ LCH, and 49 (15.6%) with MS RO+ LCH. *BRAF* was mutated in 172 patients (54.6%) with LCH.

BRAF *Status and Clinical Extent of Disease*

BRAF status of patients with LCH was related to patient characteristics, disease features, and extent of disease (Fig 2A). At diagnosis, patients with mutant *BRAF* LCH were typically younger than patients with wild-type *BRAF* (median age, 2.5 and 3.7 years, respectively; $P = .01$). Among patients with mutant *BRAF*, multisystem disease was over-represented, particularly disease with RO involvement. $BRAF^{V600E}$ mutation was found in 87.8% of patients with MS RO+ LCH, 68.6% of patients with MS RO− LCH, 43.9% of patients with SS RO− LCH, and 42.1% of patients with Lung+ LCH ($P < .001$). Among patients with LCH that involved ROs, $BRAF^{V600E}$ mutation was identified in 88.9% of patients with spleen involvement ($P < .001$), 89.2% of patients with liver involvement ($P < .001$), and 89.7% of patients with hematologic system involvement ($P < 0.001$). $BRAF^{V600E}$ mutation was apparent in 75% of patients with LCHs that involved the CNS ($P = .05$) and 72.9% of patients with LCH with pituitary gland involvement ($P = .007$). *BRAF* status was not correlated with sex or with involvement of lymph nodes, thymus, lung, or bone. In addition, $BRAF^{V600E}$ mutation was not significantly correlated with localized or multifocal bone involvement (52.6% and 54.3%, respectively; $P = .81$; Fig 2B). In contrast, skin involvement was associated with $BRAF^{V600E}$ (77.0%; $P < .001$; Fig 2A); however, few infants presented with features of localized, solitary skin SS LCH (n = 6), a rare presentation formerly called Langerhans cell histiocytoma,[21] and none were positive for $BRAF^{V600E}$. Consistent with previous reports, all six patients with solitary skin SS LCH were young infants when diagnosed (mean age at diagnosis, 1.4 months). Moreover, these patients all demonstrated spontaneous regression with no need for chemotherapy and no reactivation of disease, and median follow-up was 1.6 years. In contrast, $BRAF^{V600E}$ mutation was reported in 87.5% of patients with multifocal skin SS LCH (n = 16) and 80.2% of patients with multifocal skin MS LCH (n = 91; Fig 2B).

Mean DAS, measured at the maximum extent of LCH disease, was higher in patients with mutant *BRAF* than in those with wild-type *BRAF* (means, 3.6 and 1.4, respectively; $P < .001$). Among patients with $BRAF^{V600E}$, DAS values were high (DAS > 6) in 18.6%, intermediate (DAS, 3 to 6) in 14.5%, and low (DAS < 3) in

Table 1. Characteristics of Patients in the Studied Cohort (n = 315) Compared With Patients Not Investigated but Included in the French LCH Registry From 1983 to 2015 (n = 1,431)

Characteristic	Patients Studied for *BRAF* (n = 315)	Patients Not Studied for *BRAF* (n = 1,431)	*P*
Sex			.23
Male	53.0	56.8	
Female	47.0	43.2	
Median age at diagnosis, years	3.2	3.3	.90
HS classification			.35
SS LCH	62.2	61.8	
MS RO− LCH	16.2	19.9	
Lung+ LCH	6.0	4.7	
MS RO+ LCH	15.6	13.6	
Involvement			
Bone	81.6	83.2	.52
Skin	35.9	36.4	.90
Pituitary	15.2	14.4	.72
CNS	7.0	4.5	.09
Liver	11.8	10.3	.48
Hematologic	12.4	10.4	.31
Spleen	11.4	8.8	.16
Lung	11.8	10.3	.42
Lymph node	8.6	11.3	.16
Median follow-up, years	3.2	4.4	.003
5-year relapse	36.2	38.2	.97
Death	2.2	4.3	.11
Permanent consequence	21.0	17.8	.20

NOTE. Data are given as percentages unless otherwise noted.
Abbreviations: HS, Histiocyte Society; LCH, Langerhans cell histiocytosis; Lung +, lung involvement; MS, multiple system; RO+, risk organ involvement; RO−, no risk organ involvement; SS, single system.

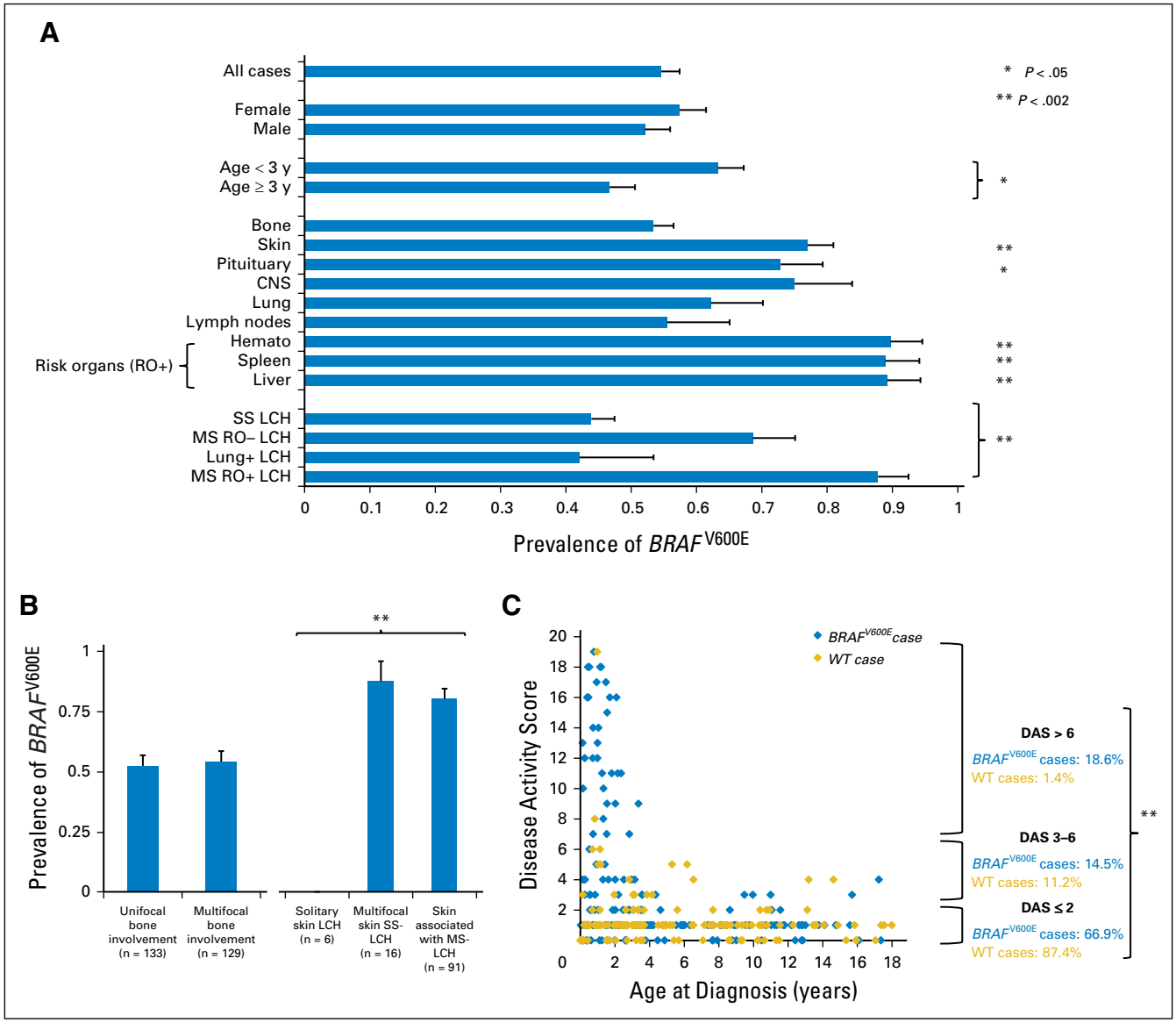

Fig 2. Clinical presentation, disease extent, and disease severity, according to *BRAF* status. (A) Prevalence of mutant *BRAF* among patients with Langerhans cell histiocytosis (LCH), according to sex, age at diagnosis, organ involvement, and extent of disease. For age, sex, and involvement of individual organs, the significance was based on comparisons between patients with and without the characteristic. For the extent of disease [lung involvement (Lung+); multiple system (MS); risk organ involvement (RO+); no risk organ involvement (RO−); single system (SS)], the significance was based on comparisons between the all four categories. (B) Prevalence of *BRAF* mutation analyzed in subgroups of patients with LCH. Among patients with bone involvement (n = 262), $BRAF^{V600E}$ prevalence was not different between patients with multifocal (54.3%) and those with unifocal (52.6%) bone lesions ($P = .81$). Among patients with skin lesions (n = 113), *BRAF* mutation was not observed in patients with localized skin lesions (solitary skin LCH), which was significantly different from the high prevalence among patients with multifocal skin lesions in SS LCH or skin lesions in MS LCH. (C) Maximum Disease Activity Score (DAS) measured during the clinical course for each patient is shown according to age at diagnosis and *BRAF* status. Blue symbols represent *BRAF*-mutated cases, and gold symbols represent *BRAF* wild-type (WT) cases. (Right) Distributions of disease severity are grouped according to three DAS ranges (≤ 2, 3 to 6, and > 6). These cutoff values are from previously published data that correlated DAS with LCH prognosis.[16] *P* values in each panel were calculated by using Fisher's exact test. Because multitests were performed, $P < .002$ was considered statistically significant. $*P < .05$; $**P < .002$.

66.9% of patients. Among patients with wild-type *BRAF* 1.4%, 11.2%, and 87.4% of patients, had high, intermediate, and low DAS, respectively (Fig 2C).

We constructed a logistic regression model with $BRAF^{V600E}$ as the dependent variable and patient age and RO involvement (ROs grouped together) as independent binary covariates. In this model (Table 2), $BRAF^{V600E}$ probability was associated only with ROs (OR, 6.35; 95% CI, 2.03 to 19.85; $P = .001$) and skin (OR, 3.65; 95% CI, 1.81 to 7.35; $P < .001$).

BRAF *Status and Biologic Parameters*

Hemoglobin level, platelet and leukocyte counts, fibrinogen, C-reactive protein, erythrocyte sedimentation rate, and albuminemia were recorded at diagnosis and at reactivation when applicable. In addition, any occurrence of hemophagocytic syndrome, according to the HLH-2004[22] criteria, was recorded. Among patients with LCH, those with $BRAF^{V600E}$ had a lower median hemoglobin level at diagnosis than did those with wild-type *BRAF* (10.1 g/dL *v* 11.8 g/dL, respectively; $P = .001$).

Table 2. Logistic Regression Analyses of Associations Between *BRAF* Status and Independent Clinical Binary Covariates

Variable	No.	OR (95% CI)	*P*
Age at diagnosis < 3 years	150	1.01 (0.57 to 1.81)	.96
Female sex	148	1.06 (0.65 to 1.75)	.80
Involvement			
Bone	262	1.52 (0.70 to 3.31)	.29
Skin	113	3.65 (1.81 to 7.35)	< .001
RO	49	6.35 (2.03 to 19.85)	.001
Pituitary	48	1.60 (0.63 to 4.08)	.32
Lung	37	0.63 (0.26 to 1.54)	.31
Lymph node	27	0.33 (0.11 to 1.01)	.05
CNS	24	1.30 (0.36 to 4.73)	.69

NOTE. Dependent variable was the *BRAF* status, and the independent covariates were patient age, sex, and potential involvement of different organs.
Abbreviations: OR, odds ratio; RO, risk organ.

Significant hypoalbuminemia, which was defined as albuminemia < 30 g/L, occurred more frequently in patients with $BRAF^{V600E}$ than in those with wild-type *BRAF* (31.0% and 9.1%, respectively; *P* = .002). Inflammatory biologic syndrome at diagnosis, defined as erythrocyte sedimentation rate > 40 mm and/or fibrinogen > 5 g/L and/or C-reactive protein > 30 mg/L, occurred more frequently in patients with $BRAF^{V600E}$ than in those with wild-type *BRAF* (20.4% and 11.2%, respectively; *P* = .03). Hemophagocytic syndrome (n = 9) was only reported in patients with $BRAF^{V600E}$.

BRAF *Status and Response to Therapy*

Among patients with LCH, 57.6% with $BRAF^{V600E}$ and 43.4% with wild-type *BRAF* were treated with a systemic chemotherapy regimen at diagnosis; this treatment was almost always a vinblastine-steroid regimen. Responses to therapy and outcome are given in Table 3. Response rates to first-line vinblastine-steroid chemotherapy were lower in patients with $BRAF^{V600E}$ than in those with wild-type *BRAF* (78.1% and 96.7%, respectively; *P* = .001).

Second-line therapy and/or rescue therapy was required in 18.6% of patients with $BRAF^{V600E}$ and in 3.5% of patients with wild-type *BRAF* (*P* < .001). In particular, second-line treatment was required in 63.6% of patients with $BRAF^{V600E}$ who had RO+ LCH. Second-line therapy was cladribine monotherapy 5 mg/m^2, administered intravenously daily for 5 days, every 28 days (n = 10 $BRAF^{V600E}$; n = 3 wild-type *BRAF*); or a combination of cladribine 9 mg/m^2/d and cytarabine 500 mg/m^2 twice daily, administered for 5 days[3] (n = 17 $BRAF^{V600E}$; n = 2 wild-type *BRAF*). Rescue therapy was an allogeneic bone marrow transplantation (n = 4 $BRAF^{V600E}$).

BRAF *Status and Reactivation, PCs, and Mortality*

Follow-up times were comparable between patients with $BRAF^{V600E}$ and those with wild-type *BRAF* (median follow-ups, 3.1 and 3.4 years, respectively; *P* = .65). Patients with $BRAF^{V600E}$ had a higher LCH reactivation risk than did those with wild-type *BRAF* (5-year reactivation rate, 42.8% and 28.1%, respectively, log-rank test; *P* = .006; Fig 3A). The difference remained significant even after exclusion of patients with RO+ LCH (5-year reactivation rate, 40.1% and 27.6%; respectively, log-rank test; *P* = .009; Appendix Table A1, online only). Reactivation in ROs was higher among patients with $BRAF^{V600E}$ than among those with wild-type *BRAF* (7.0% and 0.7%, respectively; *P* = .008; Table 3). Rate of PC was also higher among patients with $BRAF^{V600E}$ compared with those with wild-type *BRAF* (27.9% and 12.6%, respectively; *P* = .001; Table 3). Two of the main causes of PC, diabetes insipidus and neurodegenerative disease, occurred at higher rates in patients with $BRAF^{V600E}$ than in those with wild-type *BRAF* (diabetes insipidus: 19.8% *v* 8.4%, *P* = .006; neurodegenerative disease: 6.4% *v* 1.4%; *P* = .04, respectively; Fig 3B).

The 5-year mortality rate was low for both groups ($BRAF^{V600E}$, 3.9%; wild-type *BRAF*, 0.8%; *P* = .16; Appendix).

DISCUSSION

In this population-based study of 315 patients, those with a $BRAF^{V600E}$ mutation had characteristics of high-risk LCH, including an increased proportion of patients with RO involvement. $BRAF^{V600E}$ mutation was also associated with reduced sensitivity to standard LCH chemotherapy and increased rates of disease reactivation and irreversible PC.

Identification of $BRAF^{V600E}$ in more than one half of patients with LCH changed our understanding of LCH pathobiology[8]; however, previously, the clinical relevance of $BRAF^{V600E}$ remained obscure. Previous studies focused on the discovery of the mutation or its pathophysiology, but they failed to represent the full spectrum of pathology observed in this disease. Those studies concluded that $BRAF^{V600E}$ mutation occurred more

Table 3. Therapeutic Response and Outcome According to *BRAF* Status and First-Line Therapy

Outcome	All Patients			VLB Steroid Regimen			No Systemic Chemotherapy		
	BRAF	WT	*P*	BRAF	WT	*P*	BRAF	WT	*P*
All presentation at diagnosis									
Sample size, No.	172	143		96	60		73	81	
Responders, No. (%)	—	—	—	75 (78.1)	58 (96.7)	.001	—	—	—
Patient with second-line therapy, No. (%)	32 (18.6)	5 (3.5)	< .001	29 (30.2)	4 (6.7)	< .001	3 (4.1)	1 (1.2)	.35
5-year cumulative incidence of reactivations, % ± SE	42.8 ± 4.4	28.1 ± 4.5	.006	44.7 ± 5.7	37.8 ± 7.1	.14	38.3 ± 6.8	17.5 ± 5.2	.04
Reactivation in ROs, No. (%)	12 (7.0)	1 (0.7)	.008	8 (8.3)	0 (0)	.02	4 (5.5)	1 (1.2)	.19
Patients with PC, No. (%)	48 (27.9)	18 (12.6)	.001	38 (39.6)	12 (20)	.01	8 (11)	6 (7.4)	.58

NOTE. Dashes indicate not applicable.
Abbreviations: BRAF, $BRAF^{V600E}$ mutated Langerhans cell histiocytosis cases; PC, permanent consequence; RO, risk organ; SE, standard error; WT, wild-type *BRAF* Langerhans cell histiocytosis cases; VLB, vinblastine.

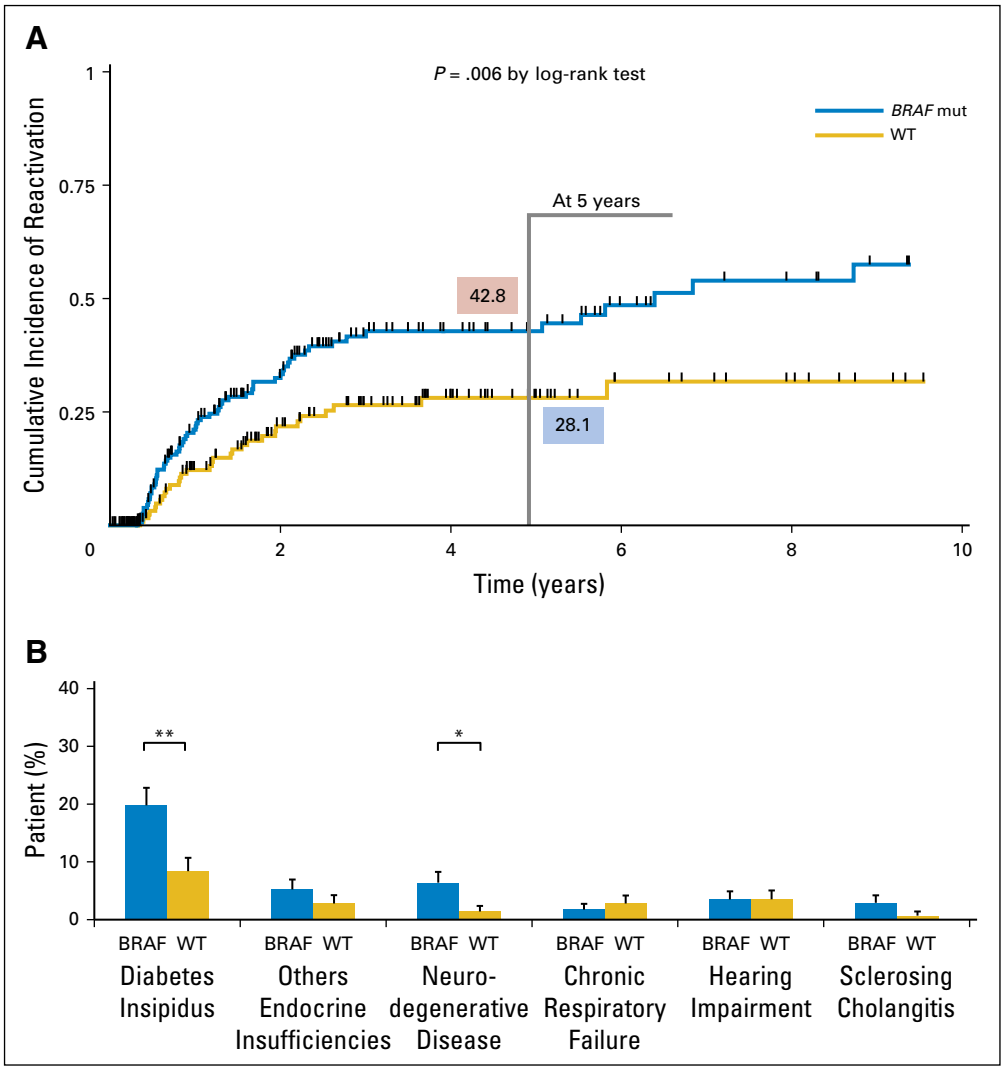

Fig 3. Outcome from Langerhans cell histiocytosis (LCH) according to *BRAF* status. (A) Cumulative incidence of reactivation curves. The green line shows the time (5 years) at which groups were compared. Boxes indicate percentages of reactivation observed in each group at that time. (B) Incidences of different types of permanent consequences (% indicates the patients in each subgroup/total patients with $BRAF^{V600E}$ mutated LCH or wild-type (WT) *BRAF* LCH. *BRAF* status is indicated as mutated (BRAF) or WT. $*P < .05$; $**P < .01$.

frequently in younger patients than in adults—on the basis of on an adult/child cohort (n=61)[8]—and that it was associated with an elevated reactivation rate, but not with RO involvement (n = 100).[7,23]

Our study enrolled children with a wide spectrum of disease phenotypes; in fact, we aimed to represent the full diversity of pathologies observed in this disease. This inclusion increased the accuracy of correlations with $BRAF^{V600E}$ expression. Our data showed that $BRAF^{V600E}$ mutation was correlated with the most aggressive forms of LCH, that is, those prevalent in patients who are diagnosed at a young age. These aggressive disease phenotypes included multisystem disease, skin involvement, spleen, liver, and hematologic dysfunction,[24] and localizations associated with PC, such as pituitary and CNS disorders.[1] Moreover, two infrequent variants of LCH segregated strictly in terms of $BRAF^{V600E}$ status. The localized LCH variant of the self-healing Hashimoto Prizker form was always found in patients with wild-type *BRAF*. In contrast, the multisystemic LCH disease with hemophagocytic syndrome–associated RO+ was always found in patients with $BRAF^{V600E}$. Apart from these two infrequent LCH variants, our results suggest that factors other than *BRAF* were likely to be involved in establishing the full LCH clinical phenotype.

One limitation of this study is that we did not investigate genotypes more fully among patients with LCH who carried wild-type *BRAF*; however, although other somatic mutations are interesting, previous correlation studies have only identified rare or largely nonrecurrent mutations in *BRAF*, *ARAF*,[25] *ERBB3*,[23] *PI3KCA*,[26] and *MAP3K1*.[27] An exception to this observation was the discovery of recurrent mutations in exon 2 and exon 3 of *MAP2K1*. Among patients with wild-type *BRAF*, these mutations—mostly deletions—affected 17% (n = 7)[23] to 27.5% (n = 11)[28] of patients with LCH. In the current study, *MAP2K1* deletions were detected in six patients with wild-type *BRAF*; these mutations were identified by Sanger sequencing of DNA from fresh frozen samples. We also detected one *PIK3CA* mutation, which was reported previously.[26] These cases were categorized as benign bone SS LCH (data not shown). Previous studies have shown that most LCH cases were associated with constitutive activation of the MAPK pathway.[8] On the basis of the clinical differences found in the current study between patients with mutated and wild-type *BRAF*, we hypothesized that in LCH, $BRAF^{V600E}$ mutation has

a stronger oncogenic potential than it has by other molecular alterations in the MAPK pathway that occurred in the presence of wild-type *BRAF*.

Association of $BRAF^{V600E}$ with a more aggressive, and sometimes resistant LCH disease, suggests an avenue for development of new therapeutic agents. LCH is an extremely heterogeneous disease, and some forms may be curable without drugs or with only minimal therapy. At the other end of the spectrum, more severe disease forms can be treated with effective second-line therapies, but these are reported to be highly toxic.[3] This toxicity is relevant, given that incidence of long-term adverse effects (PC) remains substantial for patients with LCH; more than one quarter of patients with $BRAF^{V600E}$ developed PC with LCH. Thus, anti-BRAF therapies represent a promising new line of inquiry.[29] Initial reports on anti-BRAF therapies have indicated efficacy,[11,12] but more data are needed to validate a tailored regimen that is tolerable for children, in particular, infants, who are most susceptible to high-risk LCH. Because all nine cases of LCH associated with hematophagocytic activation syndrome occurred in patients with $BRAF^{V600E}$ in our study, this association should be investigated further. Indeed, this subgroup of patients might benefit most from the addition of a targeted therapy.

In terms of improved diagnosis, $BRAF^{V600E}$ mutation can now be identified and quantified in plasma or serum-free cell DNA.[30,31] Future studies should be able to validate these assays and assess their value for prediction of prognosis and treatment response.

Patients with *BRAF* mutations who develop life-threatening histiocytoses, such as Erdheim-Chester disease and/or LCH, have been shown to manifest significant clinical responses to targeted therapy. Here, we show that children with $BRAF^{V600E}$ experienced more severe LCH disease, had higher rates of sequelae, and showed a diminished response to vinblastine-steroid chemotherapy than did children with wild-type *BRAF*. Our data argue that clinical trials should assess the benefits of *BRAF* inhibitor treatment in the early stages of disease progression.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Sébastien Héritier, Jean-François Emile, Jean Donadieu
Financial support: Jean-François Emile, Jean Donadieu
Administrative support: Sébastien Héritier, Jean-François Emile, Mohamed-Aziz Barkaoui, Jean Miron, Zofia Hélias-Rodzewicz, Jean Donadieu
Provision of study materials or patients: Caroline Thomas, Sylvie Fraitag, Sabah Boudjemaa, Florence Renaud, Anne Moreau, Michel Peuchmaur, Catherine Chassagne-Clément, Frédérique Dijoud, Valérie Rigau, Despina Moshous, Anne Lambilliotte, Françoise Mazingue, Kamila Kebaili, Eric Jeziorski, Geneviève Plat, Nathalie Aladjidi, Alina Ferster, Hélène Pacquement, Claire Galambrun, Guy Leverger, Ludovic Mansuy, Catherine Paillard, Anne Deville, Corinne Armari-Alla, Anne Lutun, Marion Gillibert-Yvert, Jean-Louis Stephan, Fleur Cohen-Aubart, Julien Haroche, Isabelle Pellier, Frédéric Millot, Brigitte Lescoeur, Virginie Gandemer, Christine Bodemer, Jean Donadieu
Collection and assembly of data: Sébastien Héritier, Mohamed-Aziz Barkaoui, Caroline Thomas, Sylvie Fraitag, Sabah Boudjemaa, Florence Renaud, Anne Moreau, Michel Peuchmaur, Catherine Chassagne-Clément, Frédérique Dijoud, Valérie Rigau, Jean Miron, Isabelle Pellier, Brigitte Lescoeur, Roger Lacave, Zofia Hélias-Rodzewicz, Valérie Taly, Frédéric Geissmann
Data analysis and interpretation: Sébastien Héritier, Jean-François Emile, Despina Moshous, Anne Lambilliotte, Françoise Mazingue, Kamila Kebaili, Eric Jeziorski, Geneviève Plat, Nathalie Aladjidi, Alina Ferster, Hélène Pacquement, Claire Galambrun, Laurence Brugières, Guy Leverger, Ludovic Mansuy, Catherine Paillard, Anne Deville, Corinne Armari-Alla, Anne Lutun, Marion Gillibert-Yvert, Jean-Louis Stephan, Fleur Cohen-Aubart, Julien Haroche, Frédéric Millot, Virginie Gandemer, Christine Bodemer, Valérie Taly, Frédéric Geissmann, Jean Donadieu
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Haupt R, Minkov M, Astigarraga I, et al: Langerhans cell histiocytosis (LCH): Guidelines for diagnosis, clinical work-up, and treatment for patients till the age of 18 years. Pediatr Blood Cancer 60: 175-184, 2013
2. Egeler RM, van Halteren AGS, Hogendoorn PCW, et al: Langerhans cell histiocytosis: Fascinating dynamics of the dendritic cell-macrophage lineage. Immunol Rev 234:213-232, 2010
3. Donadieu J, Bernard F, van Noesel M, et al: Cladribine and cytarabine in refractory multisystem Langerhans cell histiocytosis: Results of an international phase 2 study. Blood 126:1415-1423, 2015
4. Gadner H, Grois N, Pötschger U, et al: Improved outcome in multisystem Langerhans cell histiocytosis is associated with therapy intensification. Blood 111: 2556-2562, 2008
5. Haupt R, Nanduri V, Calevo MG, et al: Permanent consequences in Langerhans cell histiocytosis patients: A pilot study from the Histiocyte Society-Late Effects Study Group. Pediatr Blood Cancer 42:438-444, 2004
6. The French Langerhans' Cell Histiocytosis Study Group: A multicentre retrospective survey of Langerhans' cell histiocytosis: 348 cases observed between 1983 and 1993. The French Langerhans' Cell Histiocytosis Study Group. . Arch Dis Child 75: 17-24, 1996
7. Berres M-L, Lim KPH, Peters T, et al: BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups. J Exp Med 211:669-683, 2014 [Erratum: J Exp Med 212:281, 2014]
8. Badalian-Very G, Vergilio J-A, Degar BA, et al: Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood 116:1919-1923, 2010
9. Satoh T, Smith A, Sarde A, et al: B-RAF mutant alleles associated with Langerhans cell histiocytosis, a granulomatous pediatric disease. PLoS One 7:e33891, 2012 [Erratum: PLoS One doi:10.1371/annotation/74a67f4e-a536-4b3f-a350-9a4c1e6bebbd]
10. Haroche J, Charlotte F, Arnaud L, et al: High prevalence of BRAF V600E mutations in Erdheim-Chester disease but not in other non-Langerhans cell histiocytoses. Blood 120:2700-2703, 2012
11. Haroche J, Cohen-Aubart F, Emile J-F, et al: Dramatic efficacy of vemurafenib in both multisystemic and refractory Erdheim-Chester disease and Langerhans cell histiocytosis harboring the BRAF V600E mutation. Blood 121:1495-1500, 2013
12. Héritier S, Jehanne M, Leverger G, et al: Vemurafenib use in an infant for high-risk Langerhans cell histiocytosis. JAMA Oncol 1:836-838, 2015
13. Guyot-Goubin A, Donadieu J, Barkaoui M, et al: Descriptive epidemiology of childhood Langerhans cell histiocytosis in France, 2000-2004. Pediatr Blood Cancer 51:71-75, 2008
14. Rigaud C, Barkaoui M-A, Thomas C, et al: Langerhans cell histiocytosis: Therapeutic strategy and outcome in a 30-year nationwide cohort of 1478 patients under 18 years of age. Br J Haematol (in press)
15. Donadieu J, Rolon M-A, Thomas C, et al: Endocrine involvement in pediatric-onset Langerhans' cell histiocytosis: A population-based study. J Pediatr 144:344-350, 2004

16. Donadieu J, Piguet C, Bernard F, et al: A new clinical score for disease activity in Langerhans cell histiocytosis. Pediatr Blood Cancer 43:770-776, 2004

17. Gadner H, Minkov M, Grois N, et al: Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis. Blood 121:5006-5014, 2013

18. Donadieu J, Chalard F, Jeziorski E: Medical management of Langerhans cell histiocytosis from diagnosis to treatment. Expert Opin Pharmacother 13:1309-1322, 2012

19. Moreau S, Saiag P, Aegerter P, et al: Prognostic value of BRAF(V^{600}) mutations in melanoma patients after resection of metastatic lymph nodes. Ann Surg Oncol 19:4314-4321, 2012

20. Pekin D, Skhiri Y, Baret J-C, et al: Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. Lab Chip 11:2156-2166, 2011

21. Zunino-Goutorbe C, Eschard C, Durlach A, et al: Congenital solitary histiocytoma: A variant of Hashimoto-Pritzker histiocytosis. A retrospective study of 8 cases. Dermatology 216:118-124, 2008

22. Henter J-I, Horne A, Aricó M, et al: HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 48:124-131, 2007

23. Chakraborty R, Hampton OA, Shen X, et al: Mutually exclusive recurrent somatic mutations in MAP2K1 and BRAF support a central role for ERK activation in LCH pathogenesis. Blood 124:3007-3015, 2014

24. Broadbent V: Favourable prognostic features in histiocytosis X: Bone involvement and absence of skin disease. Arch Dis Child 61:1219-1221, 1986

25. Nelson DS, Quispel W, Badalian-Very G, et al: Somatic activating ARAF mutations in Langerhans cell histiocytosis. Blood 123:3152-3155, 2014

26. Héritier S, Saffroy R, Radosevic-Robin N, et al: Common cancer-associated PIK3CA activating mutations rarely occur in Langerhans cell histiocytosis. Blood 125:2448-2449, 2015

27. Nelson DS, van Halteren A, Quispel WT, et al: MAP2K1 and MAP3K1 mutations in Langerhans cell histiocytosis. Genes Chromosomes Cancer 54:361-368, 2015

28. Brown NA, Furtado LV, Betz BL, et al: High prevalence of somatic MAP2K1 mutations in BRAF V600E-negative Langerhans cell histiocytosis. Blood 124:1655-1658, 2014

29. Haroche J, Cohen-Aubart F, Emile J-F, et al: Reproducible and sustained efficacy of targeted therapy with vemurafenib in patients with BRAF (V600E)-mutated Erdheim-Chester disease. J Clin Oncol 33:411-418, 2015

30. Hyman DM, Diamond EL, Vibat CRT, et al: Prospective blinded study of BRAFV600E mutation detection in cell-free DNA of patients with systemic histiocytic disorders. Cancer Discov 5:64-71, 2015

31. Kobayashi M, Tojo A: The BRAF-V600E mutation in circulating cell-free DNA is a promising biomarker of high-risk adult Langerhans cell histiocytosis. Blood 124:2610-2611, 2014

Affiliations

Sébastien Héritier, Mohamed-Aziz Barkaoui, Jean Miron, and Jean Donadieu, French Reference Center for Langerhans Cell Histiocytosis, Trousseau Hospital; Sébastien Héritier, Sabah Boudjemaa, Guy Leverger, and Jean Donadieu, Trousseau Hospital, Assistance Publique–Hôpitaux de Paris; Sylvie Fraitag, Despina Moshous, and Christine Bodemer, Necker Hospital, Assistance Publique–Hôpitaux de Paris; Michel Peuchmaur and Brigitte Lescoeur, Robert Debré Hospital, Assistance Publique–Hôpitaux de Paris; Michel Peuchmaur, Université Paris Diderot, Sorbonne Paris Cité; Hélène Pacquement, Institut Curie Medical Center; Guy Leverger, Université Pierre et Marie Curie; Fleur Cohen-Aubart and Julien Haroche, Pitié-Salpêtrière Hospital, Assistance Publique–Hôpitaux de Paris; Roger Lacave, Tenon Hospital, Assistance Publique-Hôpitaux de Paris; Valérie Taly, Institut National de la Santé et de la Recherche Médicale, Unités Mixte de Recherche S1147, Centre National de la Recherche Scientifique SNC 5014, Université Paris Sorbonne Cité, Paris; Sébastien Héritier, Jean-François Emile, Zofia Hélias-Rodzewicz, and Jean Donadieu, Université de Versailles Saint-Quentin-en-Yvelines, Université Paris-Saclay; Jean-François Emile, Ambroise Paré Hospital, Assistance Publique–Hôpitaux de Paris, Boulogne-Billancourt; Caroline Thomas and Anne Moreau, Centre Hospitalo-Universitaire de Nantes, Nantes; Florence Renaud, Centre Hospitalier Régional Universitaire, Université de Lille; Anne Lambilliotte and Françoise Mazingue, Centre Hospitalo-Universitaire de Lille, Lille; Catherine Chassagne-Clément, Centre Léon Bérard; Frédérique Dijoud, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon; Kamila Kebaili, Institut d'Hémato-Oncologie Pediatrique, Lyon; Valérie Rigau, Gui de Chauliac Hospital; Eric Jeziorski, Hôpital Arnaud de Villeneuve, Montpellier; Geneviève Plat, Centre Hospitalo-Universitaire de Toulouse, Toulouse; Nathalie Aladjidi, Centre Hospitalo-Universitaire de Bordeaux, Bordeaux; Claire Galambrun, Hôpital de la Timone, Marseille; Laurence Brugières, Institut Gustave Roussy, Villejuif; Ludovic Mansuy, Brabois-Enfants Hospital, Centre Hospitalo-Universitaire de Nancy, Vandœuvre-lès-Nancy; Catherine Paillard, Centre Hospitalo-Universitaire de Strasbourg, Strasbourg; Anne Deville, Centre Hospitalo-Universitaire de Nice, Nice; Corinne Armari-Alla, Centre Hospitalo-Universitaire de Grenoble, Grenoble; Anne Lutun, Centre Hospitalo-Universitaire d'Amiens, Amiens; Marion Gillibert-Yvert, Centre Hospitalo-Universitaire de Tours, Tours; Jean-Louis Stephan, Centre Hospitalo-Universitaire de Saint Etienne, Saint Etienne; Isabelle Pellier, Centre Hospitalo-Universitaire de Angers, Angers; Frédéric Millot, Centre Hospitalo-Universitaire de Poitiers, Poitiers; Virginie Gandemer, Centre Hospitalo-Universitaire de Rennes, Rennes, France; Alina Ferster, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium; and Frédéric Geissmann, Memorial Sloan Kettering Cancer Center, New York, NY.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or jco.ascopubs.org/site/ifc.

Sébastien Héritier
No relationship to disclose

Jean-François Emile
Honoraria: Roche

Mohamed-Aziz Barkaoui
No relationship to disclose

Caroline Thomas
No relationship to disclose

Sylvie Fraitag
No relationship to disclose

Sabah Boudjemaa
No relationship to disclose

Florence Renaud
No relationship to disclose

Anne Moreau
No relationship to disclose

Michel Peuchmaur
No relationship to disclose

Catherine Chassagne-Clément
No relationship to disclose

Frédérique Dijoud
No relationship to disclose

Valérie Rigau
No relationship to disclose

Despina Moshous
No relationship to disclose

Anne Lambilliotte
No relationship to disclose

Françoise Mazingue
No relationship to disclose

Kamila Kebaili
No relationship to disclose

Jean Miron
No relationship to disclose

Eric Jeziorski
No relationship to disclose

Geneviève Plat
No relationship to disclose

Nathalie Aladjidi
No relationship to disclose

Alina Ferster
Travel, Accommodations, Expenses: Jazz Pharmaceuticals, Bayer

Hélène Pacquement
No relationship to disclose

Claire Galambrun
No relationship to disclose

Laurence Brugières
Consulting or Advisory Role: Millennium Pharmaceuticals
Research Funding: Novartis, Chugai

Guy Leverger
No relationship to disclose

Ludovic Mansuy
No relationship to disclose

Catherine Paillard
No relationship to disclose

Anne Deville
No relationship to disclose

Corinne Armari-Alla
No relationship to disclose

Anne Lutun
No relationship to disclose

Marion Gillibert-Yvert
No relationship to disclose

Jean-Louis Stephan
No relationship to disclose

Fleur Cohen-Aubart
No relationship to disclose

Julien Haroche
No relationship to disclose

Isabelle Pellier
No relationship to disclose

Frédéric Millot
No relationship to disclose

Brigitte Lescoeur
No relationship to disclose

Virginie Gandemer
No relationship to disclose

Christine Bodemer
No relationship to disclose

Roger Lacave
No relationship to disclose

Zofia Hélias-Rodzewicz
No relationship to disclose

Valérie Taly
Honoraria: Raindance Technologies, Boerhinger Ingelheim
Consulting or Advisory Role: Raindance Technologies

Frédéric Geissmann
Consulting or Advisory Role: Merck Sharp & Dohme

Jean Donadieu
No relationship to disclose

Acknowledgment

We thank the patients and their families for their participation in this study. This study was based on research from the Centre de Reference des Histiocytoses (www.histiocytose.org).

Appendix

Comprehensive Description of Experimental Plan: Sample Size Estimation and Study Organization

At the start of the study, in September 2011, we estimated the size of the sample of patients with Langerhans cell histiocytosis (LCH) that we would need to test for *BRAF*^{V600E} mutation. We estimated a sample size sufficient to observe at least a 20% difference between high-risk LCH and low-risk LCH groups, with a 5% type I error and a 20% type II error. When we considered that approximately 20% of all patients would have high-risk LCH, including lung involvement, and that 80% of patients would have low-risk LCH, we calculated that at least 313 patients must be enrolled to achieve sufficient statistical power according to the Casagrande and Pike method for unequal sized groups (Fleiss et al: Biometrics 36:343-346, 1980). All enrolled patients had been included in the French LCH registry; therefore, this study was nested within the French LCH registry. To minimize potential bias in selection of patients according to extent of disease, we asked the participating centers to propose the study to all patients that were observed locally, regardless of the extent of disease.

Survival Data

Death occurred in five patients with mutant *BRAF* and two patients with wild-type *BRAF*. Both patients with wild-type *BRAF* had multiple system with lung involvement LCH. Of those, one patient showed severe lung involvement at diagnosis and a mechanical complication that proved lethal at 4 months after diagnosis. The other patient had achieved long-term complete remission, and the fatality was unrelated to the disease. All five patients with *BRAF*^{V600E} who died had multiple system with involvement of risk organs LCH. Of those, two patients died after undergoing an allogeneic bone marrow transplantation, and three patients died of sepsis, secondary to the combined cladribine-cytarabine regimen. This low mortality rate could be explained by the efficacy of second-line therapy with the combined cladribine-cytarabine regimen. Treatment may overcome the refractory situation, but it had high toxicity.[3]

Table A1. Subgroup Analysis of Therapeutic Response and Outcome According to *BRAF* Status, First-Line Therapy, and RO Involvement

Outcome	All Patients			VLB Steroid Regimen			No Systemic Chemotherapy		
	BRAF	WT	*P*	BRAF	WT	*P*	BRAF	WT	*P*
SS, MS RO−, and Lung+ LCH at diagnosis									
Sample size, No.	139	138		64	55		73	81	
Responders, No. (%)	—	—	—	60 (93.7)	54 (98.2)	.37	—	—	—
Patients with second-line therapy, No. (%)	11 (7.9)	4 (2.9)	.11	8 (12.5)	3 (5.5)	.22	3 (4.1)	1 (1.2)	.35
5-year cumulative incidence of reactivations, % ± SE	40.1 ± 4.7	27.6 ± 4.6	.009	41.0 ± 6.7	37.6 ± 7.2	.13	38.3 ± 6.7	17.5 ± 5.2	.04
Reactivation in ROs, No. (%)	10 (7.2)	1 (0.7)	.01	6 (9.4)	0 (0)	.03	4 (5.5)	1 (1.2)	.19
Patients with PC, No. (%)	33 (23.7)	17 (12.3)	.02	24 (37.5)	11 (20)	.04	8 (11)	6 (7.4)	.58
MS RO+ LCH at diagnosis									
Sample size, No.	33	5		32	5		0	0	
Responders, No. (%)	—	—	—	15 (46.8)	4 (80)	.34	—	—	—
Patients with second-line therapy, No. (%)	21 (63.6)	1 (20)	.14	21 (65.6)	1 (20)	.14	—	—	—
5-year cumulative incidence of reactivations, % ± SE	52.3 ± 10.6	40.0 ± 21.9	.89	52.3 ± 10.6	40 ± 21.9	.89	—	—	—
Reactivation in ROs, No. (%)	2 (6.1)	0 (0)	1	2 (6.2)	0 (0)	1	—	—	—
Patients with PC, No. (%)	15 (45.5)	1 (20)	.37	14 (43.7)	1 (20)	.63	—	—	—

NOTE. Dashes indicate not applicable.
Abbreviations: BRAF, *BRAF*^{V600E} mutated LCH cases; LCH, Langerhans cell histiocytosis; Lung+, lung involvement; MS, multiple system; PC, permanent consequence; RO+, risk organ involvement; RO−, no risk organ involvement; SE, standard error; SS, single system; VLB, vinblastine; WT, wild type *BRAF* LCH cases.